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Title Page

Reproducibility of shear wave elastography of the Achilles tendon.

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Abstract:

Objective: To assess the reproducibility of shear wave elastography (SWE) measures in the Achilles tendon (AT) *in vivo*.

Materials and method: Shear wave velocity (SWV) of 14 healthy volunteers (7 males, 7 females; mean age 26.5 ± 3.8 years, mean height 171.6 ± 10.9 cm, mean Victorian Institute of Sports Assessment Achilles questionnaire (VISA-A) score 99.4 ± 1.2) was measured with the foot relaxed and fixed at 90° . Data was collected over five consecutive measures and five consecutive days.

Results: Mean SWV values ranged from 7.91 m/s - 9.56 m/s $\pm 0.27 - 0.50$ m/s. Coefficient of Variation (CV), correlations and Intra-Class Correlation Coefficients (ICC) scores ranged from 2.9% - 6.3%, 0.4 - 0.7 and 0.54 - 0.85 respectively. No significant differences were noted for longitudinal or transverse data with respect to protocol or time and no significant differences were noted for foot position in transverse data. Significant differences in SWV values were noted between foot positions for longitudinal scanning ($p = <0.05$), with a relaxed foot position providing SWV values on average 0.47m/s faster than a fixed position. Increased reproducibility was obtained with the foot relaxed. ICC between operators was 0.70 for transverse & 0.80 for longitudinal scanning.

Conclusions: Reproducible SWE measures were obtained over a 1hr period as well as a period of five consecutive days with more reliable measures obtained from a longitudinal plane using a relaxed foot position. SWE also has a high level of agreement between operators making SWE a reproducible technique for quantitatively assessing the mechanical properties of the human AT, *in vivo*.

Key Words: Elastography, Shear wave elastography, Achilles tendon, Ultrasound imaging.

Introduction:

The Achilles tendon (AT) is the strongest tendon in the body [1], yet it frequently suffers debilitating injuries [2,3]. Changes in elasticity occur as a consequence of both pathological change and healing [4,5] therefore elasticity imaging has the potential to highlight areas of degeneration aiding injury prevention [5,6] and might also be used to monitor rehabilitation [7] and provide information about when to return to activity.

However, the commonly used method of compression elastography (CE) is not reproducible, even when controlling for previous activity, foot positioning and using a homogenous age group of young, asymptomatic participants [8]. Shear wave elastography (SWE) may prove to be more useful, as SWE is independent of user skill [9], and validated against traditional tensile testing [10]. SWE provides quantitative measures of shear wave velocity (SWV), a commonly used surrogate for stiffness, by tracking the velocity of shear wave propagation [11,12]. Shear waves travel faster through harder tissue [11], therefore SWV is directly proportional to tissue stiffness [13].

Information about the reproducibility of SWE of the AT is limited. Most SWE studies assessing the AT utilise a relaxed foot position to avoid tendon stress [9,14], however, although the affect of foot position has previously been studied with CE [8], no studies have yet compared foot positions with SWE. Many SWE studies assess results from one single measurement [9,15–20], with a small number assessing two testing sessions [21,22]. Issues with SWE repeatability [21] are attributed to potential tendon and transducer movement during examination [14,21]. Ultrasound tissue characterisation (UTC) studies have suggested that exercise may cause alterations in tendon structure [23]. Despite this, no SWE studies have looked at results from more than two sessions or explicitly controlled for activity which could significantly impact results.

It is important to assess individual day to day variation in AT SWE results from a healthy population, before assessing pathological change, as distinction between normal daily variation and pathological change should be well defined prior to SWE being utilised in clinical settings. The purpose of this study was to assess the reproducibility of SWE in depicting stiffness in the AT *in vivo* and compare results obtained from different foot positions whilst controlling for prior activity.

Materials and Methods:

Participants:

Fourteen healthy volunteers (7 males, 7 females; mean age 26.5 ± 3.8 years, mean height 171.6 ± 10.9 cm, mean Victorian Institute of Sports Assessment Achilles questionnaire (VISA-A) score 99.4 ± 1.2) were recruited from the University department where the research was conducted. The volunteers each had 20 AT SWE measures taken, totaling 280 measures included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval for the study was obtained from the University of Brighton ethics committee. Inclusion criteria were male and female healthy volunteers (minimum score of 96/100 on VISA-A [25]) over the age of 18. Exclusion criteria included pregnancy, pain in the AT area, previous AT surgical intervention, known diagnosed Achilles tendinopathy, participants taking fluoroquinolone antibiotics or anomaly on ultrasound. No recruited volunteers were excluded from this study.

Both verbal and written informed consent was obtained from all participants prior to testing and participants identified their dominant leg by indicating the foot they would kick a ball with [1]. Data was collected in two testing blocks issued in a randomised order, following

our previous methodology [8]. All participants had five consecutive measurements taken in a one hour period and one measure taken every day, at the same time of day for a five day period. Participants were explicitly asked to refrain from exercise throughout the duration of testing above that required from their normal daily walking activity.

Scanning techniques:

During all measures, participants lay prone with both feet hanging clear of an examination table and an amount of ultrasound gel sufficient to maintain good contact between the ultrasound probe and the skin was applied. All measures were taken with a Siemens ACUSON S2000™ HELX EVOLUTION Ultrasound System (Siemens Medical Solutions, USA). Images were taken in both longitudinal and transverse planes on the dominant AT.

Conventional Ultrasound Technique:

Extended field of view 'SieScape' images were taken by a single operator (CP), with three years imaging experience, using a 14L5 probe to visualize the 'free' AT length, between insertion of the Achilles tendon at the calcaneus to the lowest fibres of soleus, following our previous methodology [8]. Tendon mid-point (D) was calculated as half AT length and used as the reference point for all subsequent measures to ensure all were taken at the tendon mid-point, relative to each participant.

Shear Wave Elastography:

Following conventional ultrasound, the system was placed into Virtual Touch IQ (VTIQ) mode, an acoustic radiation force based method that produces both qualitative and quantitative maps of SWV ranging between 0.5 and 10.0 m/s [26,27]. Images were obtained by the same operator (CP) using a linear-array 9L4 transducer probe. Thirteen set size and shape Regions of Interest (ROI) were placed manually in the same order on longitudinal

elastograms, at a standardised depth of 0.5cm, along the tendon length, starting proximally and working distally (See Fig 1).

Figure 1 here

Four ROI's were used for transverse scans, placed in the same order, at the same locations using standardised depths of 0.3cm and 0.4 cm (See Fig 2).

Figure 2 here.

Image quality was closely monitored throughout examination, tissue compression was avoided and quality maps in VTIQ mode were assessed to ensure images conformed to a high level of quality (quality map all green in colour). Elastograms were taken with the participant's foot in two different positions following the methodology from our previous work [8]. Firstly, what participants perceived to be a "relaxed" foot position and secondly feet were fixed using a custom made strap around the back of both feet and secured to the examination table. The strap was tightened until a 90° ankle joint position was achieved in the right ankle, measured by goniometry [19,28].

A second operator (an experienced sports medicine doctor with more than 15 years' experience in musculoskeletal ultrasound (NW)), also scanned three participants to assess ICC, the agreement in the measures between two separate operators.

Statistical Analysis:

All statistical analysis was performed using SPSS version 20 (SPSS, Chicago, Illinois). Data are presented as means \pm SD. Distribution of groups was analysed using the Shapiro-Wilk test. Coefficient of Variation (CV) and Pearson correlation analysis (r) were calculated for each combination of testing protocol (Daily or 1 hr), foot position (fixed or relaxed) and averaged over time (Measure 1, 2, 3, 4, & 5). Intra-class correlation coefficient (ICC) was

calculated to determine inter- and intra-rater reliability, ICC was calculated for each combination of measures and averaged. ICC results were classified using the following scale: 0.00 - 0.20 = Poor, 0.20-0.40 = Fair, 0.40-0.75 = Good, >0.75 = Excellent [14,29].

Standard error of measurement (SEM) was calculated as $SD * \sqrt{1 - ICC}$ and minimal detectable change (MDC) was calculated as $SEM * 1.96 * \sqrt{2}$ [30]. Differences between foot position, time and protocol were assessed by ANOVA, data was checked for sphericity and the Huynh-Feldt Correction applied if necessary. Statistical significance was defined as an alpha level of $p < 0.05$.

Results:

For each participant, SWE data was collected and averaged from 13 ROI's in the longitudinal plane and 4 ROI's in the transverse plane. Values obtained from the various SWE testing procedures are shown in table 1.

Table 1 here

The CV's for all measured variables were considered acceptably low (<12%) ranging from 2.9% - 6.3%. Correlations between measures ranged from 0.4 - 0.7 (p value range $p = 0.005$ - 0.19), all considered moderate to strong. ICC's were all considered good or excellent (range 0.54 - 0.85). Exact scores for each combination of measures are outlined in table 1 along with all other calculated measures.

ANOVA results indicated no significant differences for longitudinal or transverse data with respect to protocol or time. No significant differences were apparent in SWV results between measures collected over 1 hour and those collected over 5 days. Results did not differ significantly over time (Measure 1, 2, 3, 4 and 5). No significant differences were found for

transverse data between a fixed and relaxed foot position, indicating foot position during transverse scanning does not significantly influence results.

A significant difference was shown to exist between a fixed and relaxed foot position for longitudinal scanning ($p < 0.05$). The results indicate more repeatable and reproducible results (concluded from lower SD, SEMean, CV, TE, SEMeasure & MDC & higher R & ICC values) were obtained in the longitudinal plane from a relaxed foot position.

With regards to the ICC result, the result for transverse scanning was 0.70 (95% confidence intervals (CI) 0.030 - 0.912), classed as "good", and 0.80 (95% confidence intervals (CI) 0.624 – 0.896) for longitudinal scanning, classed as "excellent".

Discussion:

In clinical practice, the ability to form a real-time, objective and quantitative assessment of stiffness in the AT *in vivo* would be extremely useful. This study assessed the reproducibility of SWE in depicting the mechanical properties of the human AT *in vivo* using two different foot positions. The main results demonstrate no significant differences in either longitudinal or transverse data in relation to protocol, meaning that no significant differences were found in the data either when measures were taken one after the other in a 1hr time period or whether they were taken over consecutive days. This suggests that SWE has both good repeatability and good reproducibility. The other main finding was that no significant differences were found between foot positions for transverse scans, however a significant difference was shown to exist between foot positions for longitudinal scans.

Foot position was assessed to examine whether fixing the foot at 90° or allowing a relaxed foot position during SWE examination provided more reliable results. No significant differences were shown for transverse scans between the two different foot positions. A

significant difference was shown to exist between foot positions for longitudinal scans ($p < 0.05$) with a fixed foot position providing slower SWV values of approximately 0.45m/s. As a slower SWV relates to softer tissue, it is recommended that foot position remains consistent when comparing scans. In the longitudinal plane, a relaxed foot position produced more reliable results, concluded from lower SD, SEMean, CV, TE, SEMeasure and MDC scores together with higher R and ICC scores. The use of a relaxed foot position would agree with previous research findings that it avoids tendon stress [35] and provides additional support for imaging of the AT to be conducted in a resting position [19]. This study suggests longitudinal SWE scans provide more reliable measures than transverse, and furthermore, in the longitudinal plane, a relaxed foot position would be advisable.

For both longitudinal and transverse scanning, over both protocols, there were no significant differences noted in time, suggesting the five separate measures were not significantly different from one another. This was an expected finding as large variations in SWV would not be expected over short periods of time. This agrees with previous research showing no significant differences in the mechanical properties of the free AT between days [36].

SWE functions independently of operator skill [9], however there is a lack of research into consistency of measures obtained by different operators using SWE to image the AT. To address this and replicate a more realistic use of SWE in the clinical setting where more than one clinician may perform scans, a second operator (NW) assessed inter-rater reliability. The ICC result for transverse scanning was 0.70, classed as good and 0.80 for longitudinal scanning, classed as excellent. Agreement between different operators was considered high, especially for longitudinal scanning in agreement with previous research [9].

SWE research often displays results in Young's modulus (E), however for this study to do so would rely on conversion of SWV to E using the equation $E = 3pv^2$ where v is SWV and p is

tissue density [11]. This equation assumes tissue isotropy based on 1000 kg.m^3 used as a constant tissue density, however this may not always be true for anisotropic tendon [31]. Therefore, values in this study (and others [19]), were reported as SWV.

Medical literature shows no consistency in choice of reliability calculations [32], however SWE literature commonly uses ICC as a sole reliability measure [17,20,21,33]. The ICC results gained from this study ranged between fair and good, and are supported by additional analysis to strengthen any conclusions drawn. CV's have also been used as a measure of repeatability [34] with a value below 12% considered acceptably low for a biological measure [29]. The CV values in this study were all considered acceptably low, ranging between 2.9% - 6.3%, supported by moderate to strong correlations between measures. These findings suggest SWE is a reproducible technique for the quantitative assessment of the AT, supporting the view that SWE can provide clinicians with additional functional information on tendon health [9].

Initial data analysis demonstrated transverse scans returned significantly different SWV values than longitudinal scans. Transverse scans with a relaxed foot position provide SWV values $1.63 \pm 0.535 \text{ m/s}$ slower than longitudinal scans ($p=0.000$). With a fixed foot position, SWV from transverse scans return a SWV $1.11 \pm 0.622 \text{ m/s}$ slower than longitudinal measures ($p=0.000$). The differences in the noted velocities between transverse and longitudinal scans were attributed to shear waves propagating along the length of fibres more easily than they do across them [10], therefore longitudinal scans produce faster SWV's. As significant differences were noted between longitudinal and transverse scans, further examination revealed more reliable data (concluded from lower SD, SEMean, CV, and higher R values) were obtained from the longitudinal scans in normal healthy tendons. Future work is needed to clarify the best protocols to use where pathology is concerned, which is outside the scope of this study.

Related research noted many factors can potentially influence the repeatability of SWE, with movement being the primary concern. One such study found SWE had only fair repeatability, however many methodological differences between these two studies [21] could explain these differences. The studies used machines from different manufacturers, yet comparison of results across different machines and manufacturers must be made with caution as each has its own nuances to reach their goal [37]. The other study [21] only reported ICC values (poor on right ICC = 0.17, good on left ICC = 0.62, with global ICC considered 'moderate' ICC = 0.42). In contrast, ICC's from our study were in the good or excellent category, ICC results were supported by other measures and rather than examining participants bilaterally, this study only assessed dominant side, due to differences being noted in tendon properties of dominant and non-dominant legs [1]. There were also differences in scan location, with mid portion chosen as the point of interest in this study due to regular occurrence of Achilles tendinopathy in this area [38,39].

The main limitation of this research is its relatively small sample size, yet despite this, each of the fourteen volunteers had 20 measures taken, totaling 280 measures included for analysis, a number much higher than comparable research [9,15,21]. This study is a novel examination, providing an initial step in the assessment of SWE in the tendon imaging setting and future research with larger cohorts should be undertaken. This study only assessed SWE in relation to the AT and it would be interesting to see results from other injury prone areas in the body such as the patella or supraspinatus tendons or lateral epicondyle [40].

Since the advent of SWE, it is now possible to perform real-time, *in vivo* measurements of tendon stiffness which could improve our knowledge of the AT as well as tendinopathy development and patterns of rehabilitation. In comparison to compression elastography, SWE shows a much higher level of reliability for obtaining measurements of stiffness from

the AT. The application of SWE for detecting subtle changes in tendon stiffness associated with rehabilitation protocols should also be assessed in future studies.

The results of this study indicate that SWE is a reproducible technique for quantitatively assessing the mechanical properties of the human AT, *in vivo*. The results offer novel findings in the use of SWE for AT assessment and suggest that longitudinal scans and a relaxed foot position offer the most reproducible results. Consistent results can also be gained over different days with high levels of agreement between different operators.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Figure Legends:

Fig 1: Figure 1: Longitudinal elastogram of the Right Achilles tendon taken from a 27 year old female participant. The boundary of the Achilles tendon is denoted by the white lines along the top of the image. The 10 Regions of Interest (ROI's) used to collect SWE data when imaging in the transverse plane image are shown by the yellow squares. The corresponding SWE values in m/s are shown to the right of the image and are highlighted in the red circle.

Fig 2: Figure 2: Transverse elastogram of the Right Achilles tendon taken from a 30 year old male participant. The boundary of the Achilles tendon is denoted by the white circle. The 4 Regions of Interest (ROI's) used to collect SWE data when imaging in the transverse plane image are shown by the black circles. The corresponding SWE values in m/s are shown in the left of the image and highlighted in the red circle.

Tables:

Table 1: All calculated analysis on SWE results for all participants over all protocol and foot position combinations. Data includes standard deviation (SD), Standard Error of the Mean (SEMean), Coefficient of Variation (CV), Pearson product-moment correlation coefficient (R), Intra-Class Correlation Coefficient (ICC), Typical Error (TE), Standard Error of the Measurement (SEMeasure) & Minimal Detectable Change (MDC).

Table 1:

	Daily Measures				1 Hour Measures			
	Longitudinal		Transverse		Longitudinal		Transverse	
	Fixed	Relaxed	Fixed	Relaxed	Fixed	Relaxed	Fixed	Relaxed
Mean (m/s)	9.04 ±	9.56 ±	7.96 ±	7.93 ±	9.12 ±	9.54 ±	7.98 ±	7.91 ±
± SD	0.44	0.27	0.31	0.48	0.47	0.29	0.35	0.50
SEMean	0.12	0.07	0.08	0.13	0.13	0.08	0.09	0.13
CV	4.9%	2.9%	3.9%	6.0%	5.2%	3.1%	4.4%	6.3%
R	0.6	0.4	0.5	0.6	0.5	0.4	0.6	0.7
ICC	0.71	0.54	0.62	0.71	0.67	0.55	0.78	0.85
TE	0.31	0.20	0.14	0.25	0.33	0.23	0.15	0.19
SEMeasure	0.23	0.19	0.19	0.26	0.27	0.20	0.17	0.19
MDC	0.65	0.51	0.53	0.71	0.76	0.55	0.46	0.53

SD = Standard Deviation, SEMean = Standard Error of the Mean, CV = Coefficient of Variation, R = Pearson product-moment correlation coefficient, ICC = Intra-Class Correlation Coefficient, TE = Typical Error, SEMeasure = Standard Error of the Measurement, MDC = Minimal Detectable Change.

Correlations: 0 = no correlation, 0.1-0.3 = weak, 0.4-0.6 = moderate, >0.7 = strong and 1 = perfect [41]

ICC results: 0.00 - 0.20 = Poor, 0.20-0.40 = Fair, 0.40-0.75 = Good, >0.75 = Excellent [14,29].

Figures:

Fig 1:

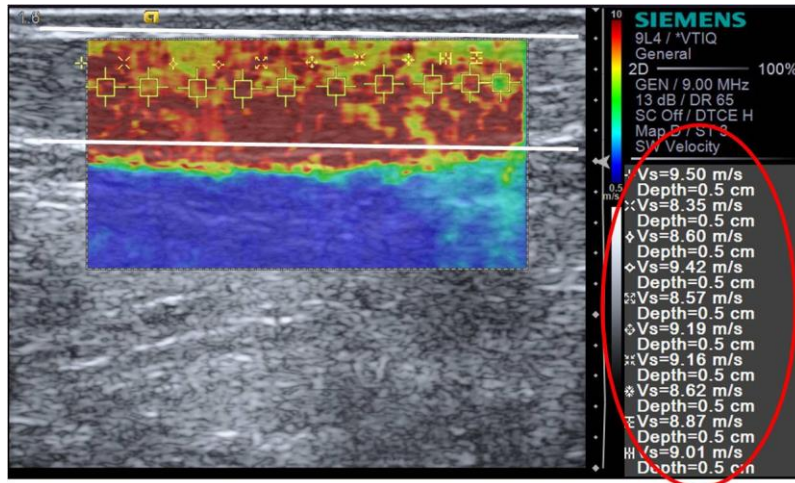


Fig 2:

